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Sugars Production by Bacteria and Actinomycetes Isolated from Soil, Rhizosphere and Mycorrhizosphere of Pine (*Pinus sylvestris L.*)

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Abstract

It was found that the bacteria studied did not excrete sugars into the medium but rather utilized the sugars (glucose and fructose) as impurities of sodium gluconate. Nevertheless in post-culture media of soil and rhizosphere bacteria, traces of sugars like: ribose, arabinose and an unidentified sugar Z_3 were detected. More actinomycetes strains originating from the soil than those from the mycorrhizosphere released sugars into the media.

Introduction

In soil among different organic compounds, simple sugars and oligosaccharides are present (Greenland and Oades, 1975). They are products of polysaccharides' hydrolysis (of plant and animal origin) — (Trojanowski, 1973). An important source of these compounds are also plant root exudates (Rovira, 1965).

Sörensen and Paul (1971) observed transformation of acetic acid added to soil into amino acids and simple sugars. However it is not known whether these compounds were excreted by living cells of soil microorganisms or were released from the dead ones as autolysis' products.

Excretion of simple sugars, their derivatives and oligosaccharides by saprophytic microorganisms seems to be of special importance in the mycorrhizosphere, since these compounds are practically the only easily available carbon and energy sources for ectomycorrhizal fungi (Palmer and Hacskaylo, 1970; Hacskaylo, 1973).

Beside the host plant also the accompanying microorganisms could be the source of these compounds for the mycorrhizal fungi. Therefore these studies were undertaken.

Experimental

Materials and Methods

Forty nine strains of bacteria (identical or similar to the genus *Arthrobacter*) and seventy five strains of actinomycetes (*Streptomyces sp.*) isolated from soil, rhizosphere and mycorrhizosphere of pine were used in present studies.

The bacteria and actinomycetes were grown for 7 days at 26°C in the same medium as used in studies on organic acids production (Różycki, 1985; Różycki and Strzelczyk, 1986) but sodium gluconate (5 g/litre) instead of glucose was used.

The bacteria were removed from the medium by centrifugation (15.000 r.p.m., for 20 min) or filtration on filter paper (actinomycetes). The post-culture liquids were desalted by shaking (on the shaker) with ion exchange resins (3 g of the resin/50 ml of post-culture liquid): — with Dowex 50 W X8 (H^+ form, 100—200 mesh) — for 2 hours, and then after filtration — with Dowex 1 X8 (CO_3^{2-} form, 100—200 mesh) — for 2 hours. Subsequently the ion exchange resins were regenerated: Dowex 50 — with 2 M HCl and Dowex 1 — successively with 1 M NaOH and with 1 M Na_2CO_3; and washed with distilled water to neutral reaction and dried.

The desalted post-culture liquids after separation of the anion exchange resin by filtration on filter paper were evaporated at 50°C in an oven with air circulation and the dry residue obtained was dissolved in 0.5 ml of 10% isopropanol.

In such samples the total pool of reducing sugars was estimated by Somogyi-Nelson's method (Nelson, 1944). Into test tubes (in 3 replications) 0.05 ml of sample studied, 1 ml of distilled water and 1 ml of Samogyi's copper reagent were added and was kept in boiling water bath for 20 min. The samples were then brought to room temperature and 1 ml of Nelson's arsenate-molybdate reagent and 10 ml of distilled water were added. After mixing, the extinction was measured in the spectrophotometer Spekol ZV (C. Zeiss, Jena, G.D.R.) at the wavelength 520 nm against blank sample prepared from the same reagents but with 0.05 ml of 10% isopropanol instead of the sample studied. Amounts of reducing sugars were read on the basis of extinetion values from the standard curve for glucose.

The separation and identification of the sugars in the same samples was performed by the method of ascending paper chromatography. 40 µl of the sample or standarts (1% solutions of rafinose, maltose, saccharose, glucose, fructose, arabinose, xylose and rhamnose) were spotted on the filter paper — Whatman No. 1 (46 × 46 cm) and then the chromatograms were developed in one direction (four-fold successive development and drying) in the solvent system: ethyl acetate — isopropanol — water (65:23:12, v/v/v). The colour development on the chromatograms was achieved by dipping them in the following reagent: 5 parts of 2% ethanolic solution of aniline, 5 parts of 2% ethanolic solution of diphenylamine and 1 part of 85% H_3PO_4 (v/v/v, mixed immediately before use) and by heating at 80°C for 5 min. Aldohexoses were coloured on gray-blue; ketoses (fructose) — on olive-brown and pentoses — on gray-green (Clotten and Clotten, 1962).

The results of preliminary studies have shown that sodium gluconate used (pure, "Fluka") contained the relatively large amounts of glucose and fructose. In order to state whether or not in post-culture liquid is statistically significant less (the utilization) or more (the excretion) of reducing sugars than in the control, analysis of variance and L.S.D. ($P = 0.05$) were applied. The same statistical tests were used for comparison of mean concentrations of reducing sugars in post-culture liquids of microorganisms from different isolation sources (soil, rhizosphere, mycorrhizosphere).

Results

The results of our studies are presented on Fig. 1—2 and in Tables I—III. It appears from Fig. 1 that the bacteria did not release sugars into the medium, but in different degrees utilized these compounds (glucose and fructose) present as impurities of sodium gluconate.

Chromatographic analysis of the post-culture fluids of bacteria only in few cases revealed the presence of small amounts of compounds other

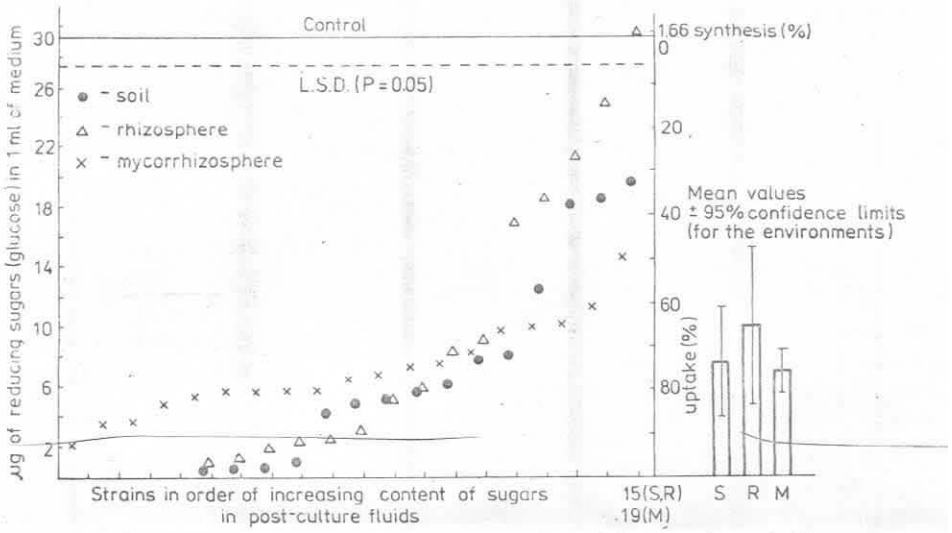

Fig. 1. The concentration of sugars in bacterial post-culture fluids.
Histograms present the mean values for: S — soil, R — rhizosphere and M — mycorrhizosphere strains.

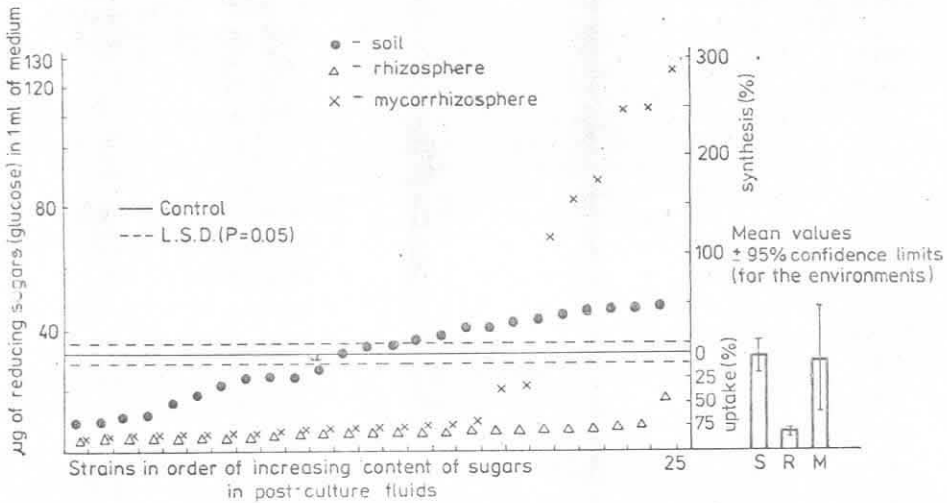

Fig. 2. The concentration of sugars in actinomycetes post-culture fluids.
Explanations as in Fig. 1.

Table I

R_{Rh} coefficients (R_f in relation to rhamnose) and colours of the spots of sugars

Sugars	R_{Rh}	Colours of the spots
Rhamnose	1.00	olive-green
Ribose	0.88	gray-green
Xylose	0.67	gray-green
Arabinose	0.54	gray-green
Fructose	0.46	orange-brown
Glucose	0.32	gray-blue
Galactose	0.26	gray-blue
Sacharose	0.11	brown
Maltose	0.06	blue
Rafinose	0.01	gray
Z_1	0.18	red-brown
Z_2	0.36	blue-violet
Z_3	0.89	yellow-green
Z_4	0.81	orange-brown

than glucose and fructose (Table II). In the culture supernatants of several strains from soil and rhizosphere traces of arabinose, xylose, an unidentified sugar Z_3 and ribose were detected. In post-culture liquids of bacteria

Table II

Sugars in post-culture fluids of the bacteria

Source of isolation	Sugars				Number of strains studied
	Arabinose	Xylose	Ribose	Z_3	
Soil	2 (13.3)	0	2 (13.3)	3 (20.0)	15
Rhizosphere	1 (6.7)	3 (20.0)	0	3 (20.0)	15
Mycorrhizosphere	0	0	0	0	19

Explanation: per cent — given in paranthesis.

isolated from the mycorrhizosphere no sugar was detected apart from glucose and fructose — present also in the control (Table II).

It appears from Fig. 2 that almost half of the soil actinomycetes (44%) significantly utilized the sugar-impurities and just as many strains from soil released sugars. All the actinomycetes isolated from the rhizosphere utilized the sugars present in the medium. The same was stated in 76% of the organisms derived from the mycorrhizosphere. The remaining 24% of mycorrhizosphere strains excreted considerable amounts of reducing sugars into the medium (Fig. 2).

In the culture filtrates of soil actinonycetes (unidentified compounds: Z_1, Z_2, Z_3 and arabinose and xylose were detected. Also trace amounts

Table III

Sugars in post-culture fluids of the actinomycetes

Source of isolation	Sugars							Number of strains studied
	Z_1	Z_2	Arabinose	Xylose	Z_3	Z_4	Others*)	
Soil	8 (32)	22 (88)	11 (44)	17 (68)	19 (76)	0	0	25
Rhizosphere	0	0	6 (24)	2 (8)	0	0	0	25
Mycorrhizo-sphere	0	0	0	2 (8)	0	6 (24)	6 (24)	25

Explanations: — see footnote in Table II; *) — 4—7 of different unidentified sugars in post-culture fluids of given strain having R_{Rh} from 0.28 to 1.15

of arabinose and xylose in post-culture liquids of some root-zone strains were noted. However the other mycorrhizosphere strains (24%) excreted into the medium in very large amounts an unidentified sugar Z_4 and other unidentified compounds (Table III).

Discussion

Data on the production of simple sugars and their derivatives by soil microorganisms are scare. However the importance of sugars in mycorrhizal associations is known (Hacskaylo, 1973). It is known that symbiotic fungi of forest trees are capable of utilizing almost exclusively sugars supplied by roots of the plant (Lewis and Harley, 1965; Palmer and Hacskaylo, 1970; Hacskaylo, 1973; Lamb, 1974; Richards, 1974; Nylund, 1981). The excretion of these compounds by mycorrhizosphere microorganisms may be an additional source of sugars for the symbionts.

Nevertheless, although the bacteria did not release sugars into the medium, in the post-culture fluids traces of compounds other than present in the control were detected chromatographically. It can be supposed that the release of these compounds was a result of the cell autolysis.

We have used the medium applied in this work, because most of the microorganisms studied grew better in the presence of sodium gluconate then in other media. Furthermore gluconic acid was produced in large amounts by the pleomorphic bacteria from the root zone — mainly from the mycorrhizosphere (Różycki, 1985).

In the present studies sugars excretion was stated only in cultures of actinomycetes. The relase of sugars was found only in strains similar to *Streptomyces longispororuber* (from the soil) and to *S. griseus* (from the mycorrhizosphere). The capability of production of these compounds by actinomycetes was observed less often than amino acids and organic acids biosynthesis (Różycki and Strzelczyk, 1985, 1986). Therefore it is difficult

to assess a true ecological importance of carbohydrates' excretion by microorganisms. Moreover almost nothing is known about the capability of sugars production by fungal mycelium. It is difficult to state whether mannitol excretion by mycorrhizas was a results of the synthesis of this compound by the mycelium mantle or this was a result of mycelium autolysis (Hassoumo and Wareing, 1964 — c.f. Foster and Marks, 1967; Lewis and Harley, 1965; Foster and Marks, 1967). Further studies on sugars production (and on other organic compounds) by microorganisms associated with ectomycorrhizas and by symbiotic fungi are needed for better understanding of metabolic interactions in the associations: plant — fungus — mycorrhizosphere microorganisms.

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